Final Report

Washington State Recreational Guidance for Microcystins (Provisional) and Anatoxin-a (Interim/Provisional)

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Introduction

Cyanobacteria (also known as blue-green algae) are found in water bodies throughout Washington. When environmental conditions are favorable, cyanobacteria can grow rapidly, often creating a bloom or surface scum. Some conditions that influence bloom formation are nutrient availability, light intensity, water temperature, species competition, wind patterns, water column mixing, and zooplankton predation. In some cases, cyanobacteria produce toxins. Although environmental factors leading to toxin production are not well understood, we do know that toxins are more likely to reach harmful concentrations during bloom conditions. Only laboratory tests can confirm if a bloom is toxic or non-toxic.

Numerous species of cyanobacteria are capable of producing several types of toxins, including hepatotoxins (liver toxins), neurotoxins (nerve toxins), as well as dermal and gastrointestinal irritants. These substances can be toxic to humans, pets, and wildlife. At present, there are no federal recreational standards for cyanobacterial toxins in the U.S.

Toxic cyanobacterial blooms in Washington ponds, lakes, and reservoirs have been documented at an increasing rate over the past 25 years. Periodic reports of pet illnesses and death have recently increased. As a result of citizen concerns, Ecology's Water Quality Program was directed and funded by the legislature to create a Freshwater Algae Control Program. Stakeholders directed Ecology to prioritize a statewide program to test bloom samples for toxicity and to develop recreational guidelines to direct local health jurisdictions (LHJs) in implementation efforts. This document offers recreational guideline values (provisional) for microcystins and anatoxin-a (interim/provisional) and a tiered framework to follow in the event of a toxic occurrence.

Cyanobacteria of Concern

Washington State Department of Health (DOH) has identified a list of cyanobacteria genera and species of concern for lakes in Washington. If the following genera are identified in a water sample from an algal bloom, the sample should be tested for toxicity:

- *Microcystis*
- *Anabaena*
- *Aphanizomenon*
- *Gloeotrichia*
- *Oscillatoria/Planktothrix*
- *Cylindrospermopsis*
- *Lyngbya*
- *Nostoc.*

Gloeotrichia is included in this list because exposure to this genus has lead to reports of human health impacts in Washington lakes. A recent study identified microcystin-LR production by *Gloeotrichia echinulata* (Carey et al. 2007).

The World Health Organization (WHO 1999) identifies several other genera as capable of producing toxins (Table 1). These species are less likely to be observed in Washington water bodies. They include:

- *Anabaenopsis, Hapalosiphon, Nodularia, Schizothrix, Umezakia*.

Scientists believe that some toxic cyanobacteria remain unidentified. Further, it is likely that many cyanobacterial toxins have not yet been discovered.

Cyanobacterial Toxins and Symptoms

Cyanotoxins are a diverse group of natural toxins that fall into three broad chemical structure groups (Table 1). These are cyclic peptides (microcystins and nodularin), alkaloids (anatoxins, saxitoxins, cylindrospermopsin, aplysiatoxins and lyngbyatoxin), and lipopolysaccharides (irritants). This guidance addresses microcystins and anatoxin-a.

Table 1. World Health Organization (WHO) list of toxic cyanobacteria.

Toxin group	Primary target organ in mammals	Cyanobacterial genera
Cyclic peptides		
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis*
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Anatoxin-a(s)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomemon, Umezakia*
Lyngbyatoxin-a	Skin, gastrointestinal tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LGS)	Potential irritant; affects any exposed tissue	All

From: WHO. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. Edited by Ingrid Chorus and Jamie Bartram. E & FN Spon. London and New York.

Microcystins

Microcystins are the most thoroughly investigated cyanobacterial toxins (Falconer 2005). At least 71 structural variants have been identified, and microcystin-LR is the variant most commonly found in cyanobacteria (Huisman et al. 2005, Botana 2007). Microcystins have been identified in *Anabaena*, *Microcystis*, *Oscillatoria* (*Planktothrix*), *Nostoc*, and *Anabaenopsis* species and from the terrestrial genus *Hapalosiphon* (WHO 1999). More than one microcystin may be found in a particular cyanobacteria strain.

Microcystins are cyclic heptapeptides that primarily affect the liver in animals. A lethal dose of microcystins in vertebrates causes death by liver necrosis within hours or up to a few days. Microcystins block protein phosphatases 1 and 2A (important molecular switches in all eukaryotic cells) with an irreversible covalent bond (MacKintosh et al. 1990 in WHO 2003). Liver injury is likely to go unnoticed and results in (external) noticeable symptoms only when it is severe (WHO 2003). Other studies have shown that microcystin toxicity is cumulative (Fitzgeorge et al. 1994). Researchers suspect microcystins are liver carcinogens, which could increase cancer risk to humans following continuous, low level exposure.

Symptoms of Microcystin Exposure

Symptoms of microcystin poisoning may take 30 minutes to 24 hours to appear, depending upon the size of the animal affected and the amount of toxin consumed. Gross and histopathologic lesions caused by microcystins are quite similar among species, although species sensitivity and signs of poisoning can vary depending on the type of exposure. One of the earliest effects (15-30 minutes) of microcystin poisoning is increased serum concentrations of bile acids, alkaline phosphatase, γ-glutamyltransferase, and AST. Microcystin symptoms in mammals and other animals may include jaundice, shock, abdominal pain/distention, weakness, nausea/vomiting, severe thirst, rapid/weak pulse, and death. It is likely that the number of incidents with low-level symptoms such as nausea, vomiting, and diarrhea associated with recreational exposure to cyanobacterial toxins are under reported. Death may occur following exposure to very high concentrations within a few hours (usually within 4-24 hours) or up to a few days. Death is due to intrahepatic hemorrhage and hypovolemic shock. In animals that live more than a few hours following high level exposure, hyperkalemia or hypoglycemia, or both, may lead to death from liver failure within a few days (Merck Veterinary Manual 2008 online at: http://www.merckveterinarymanual.com/mvm/index.jsp?cfile=htm/bc/210200.htm).

According to the Merck Veterinary Manual, surviving animals have a good chance for recovery because the toxins have a steep dose-response curve. Activated charcoal oral slurry is likely to benefit exposed animals, even though therapies for cyanobacterial poisonings have not been investigated in detail. The link states that an ion-exchange resin such as cholestyramine has proved useful to absorb the toxins from the GI tract and that certain bile acid transport blockers such as cyclosporin A, rifampin, and silymarin injected before dosing of microcystin have been effective in preventing hepatotoxicity in laboratory studies.

Anatoxin-a

Anatoxin-a is one of three neurotoxic alkaloids that have been isolated from cyanobacteria (Falconer 2005). It is produced by various species of cyanobacteria including *Anabaena*, *Planktothrix* (*Oscillatoria*), *Aphanizomenon*, *Cylindrospermum*, and *Microcystis* spp. Anatoxin-a was first detected in Canada in the 1960s (Gorham et al. 1964). Between 1961 and 1975, cattle and dog poisonings associated with *Anabaena flos-aquae* blooms occurred in six locations in Canada. Since then, most anatoxin-a detections have been in Europe, and, outside Europe, in North America (Botana 2007).

Anatoxin-a is a bicyclic secondary amine that mimics the neurotransmitter acetylcholine and binds to the nicotinic acetylcholine receptor at the axon terminal at the neuromuscular interface (Botana 2007, Huisman et al. 2005). Binding of anatoxin-a is irreversible; the sodium channel is locked open, becomes overstimulated, fatigued, and eventually paralyzed. In the respiratory system, anatoxin-a exposure results in a lack of oxygen to the brain, subsequent convulsions, and death by suffocation. Anatoxin-a is about 20 times more potent a nicotinic agonist than acetylcholine (Botana 2007).

A methylene analogue of anatoxin-a called homoanatoxin-a was isolated from *Planktothrix formosa* in Norway. Symptoms of homoanatoxin-a are similar to those observed with anatoxin-a. This analogue has been found recently in Ireland and Japan (Botana 2007).

Alkaloid toxins are more likely to be present in free water than the cyclic peptide toxins microcystins and nodularin (WHO 2003). While microcystins appear to be more common than neurotoxins, neurotoxins are more potent and have caused severe animal poisonings in North America, Europe, and Australia (WHO 2003). Anatoxin degrades readily to nontoxic degradation products in sunlight and at a high PH (Botana 2007). In natural blooms in eutrophic lakes, anatoxin-a half-life is typically less than 24 hours, while the half-life in the laboratory was about five days (WHO 1999). The rapid degradation of anatoxin-a presents problems with determining toxin levels after exposure. According to Botana (2007), samples should be protected from light and acidified prior to storage at -20^{o}C in order to limit anatoxin-a degradation.

Symptoms of Anatoxin-a Exposure

Neurotoxins are notoriously rapid acting poisons; anatoxin-a was originally called very fast death factor (VFDF) due to its potency (Botana 2007). Illness and death to an animal may occur within a few minutes to a few hours after exposure, depending on the size of the animal and amount of toxic bloom consumed. An animal with anatoxin-a toxicosis may exhibit staggering, paralysis, muscle twitching, gasping, convulsions, backward arching of neck in birds, and death.

Livestock that drink large amounts of contaminated water and pets that collect scum on their fur then ingest it by licking are at highest risk from anatoxin-a exposure. While anatoxin-a is largely retained within cells when conditions for growth are favorable, toxins will be liberated in the gastrointestinal tract if water containing toxic cells is consumed (WHO 1999, Botana 2007). However, ingestion of a sublethal dose of these neurotoxins leaves no chronic effects and

recovery appears to be complete with no ongoing injury (WHO 2003). Exposure leaves no sign of organ damage and residual toxin is rapidly degraded (Botana 2007).

Exposure Pathways

The most likely exposure pathways to microcystins and anatoxin-a are through recreational contact, contaminated drinking water, and ingestion of dietary blue-green algae supplements (which are derived from freshwater algae). Long-term chronic ingestion via drinking water and exposure through consumption of fish and shellfish are not considered in the following exposure scenario. This effort focuses on recreational exposure, which includes activities such as swimming, wind surfing, jet skiing, and water skiing. For this assessment, DOH assumes that a swimmer or other lake user ingests 0.05 liters of water per hour and that exposure lasts for two hours per day per year.

Microcystin Risk Levels and Standards

While there is no U.S. recreational guideline for microcystins, risk from recreational exposure has been addressed in two states: Oregon and Vermont (Stone and Bress 2007). In addition, WHO has published guidelines for drinking water and recreation. The three approaches are summarized below.

Oregon Guidelines

In 2005, Oregon adopted two mechanisms for issuing advisories for recreational waters. In the first, a lake is immediately posted upon the identification of visible scum dominated by potentially toxigenic cyanobacteria. The second mechanism uses cell densities to trigger advisories. A lake is posted if the cell density of total toxigenic cyanobacteria equals or exceeds 100,000 cells/mL, unless the bloom has *Microcystis* or *Planktothrix* species, in which case the lake would be posted if there are 40,000 cells/mL. The guidance value associated with *Microcystis* or *Planktothrix* cell counts used by Oregon is estimated to correlate with the production of 8 µg/L microcystin. In Oregon, several water bodies are monitored regularly for toxic blooms and a press release is issued to media outlets when a water body is posted.

Vermont Guidelines

In Vermont, the State Department of Health and the University of Vermont collaborate in monitoring Lake Champlain for cyanobacteria, microcystins, and anatoxin-a. Peak concentrations occur in August and are heaviest in one lake bay. Vermont has a tiered system, calling for beach closings with the visible presence of cyanobacterial scum. Beaches reopen if no visible scum is present and the concentration of microcystin-LR is 6 µg/L or less. Vermont uses two types of postings: an informational poster to raise awareness of cyanobacteria and scum formation, and a "Beach Closed" sign for public swimming beaches.

World Health Organization Guidelines (WHO)

WHO has provided a provisional guideline for microcystin-LR in drinking water (0.001 mg/L) and a tolerable daily intake (provisional) of 0.04 µg/kg-day (Table 2). The TDI was based on liver pathology observed in a 13-week study in mice, with an uncertainty factor of 1000. For recreational guidance, WHO developed microcystin concentrations corresponding to low (4µg/L), moderate (20 µg/L), and high (scums) risk associated with cyanobacteria densities (Table 2).

Table 2. World Health Organization **(**WHO) guidelines and risk levels for microcystin.

	Microcystin concentration	**Cyanobacteria cells/ml**
Tolerable Daily Intake (provisional)	0.04 µg/kg-day	
Recreational Bathing Waters		
Low risk	4 µg/L	20,000
Moderate risk	20 µg/L	100,000
High risk		Scums
Drinking Water (provisional)	1 µg/L	

Washington Recreational Guidance Values: Microcystins

For Washington, DOH recommends the use of a recreational guidance value for microcystins (provisional) calculated as follows:

$$\textbf{Guidance value (µg/L)} = \frac{\textbf{TDI X BW}}{\textbf{IR}}\textbf{,}\ \text{where}$$

TDI = 0.04 µg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The tolerable daily intake (TDI) used in the above equation was developed by WHO based on microcystin-LR orally administered to mice, with observed effects on the liver. This limit accounts for chronic exposure to microcystin, including daily swimming, incidental ingestion, and inhalation through sinus passages. The resulting (provisional) recreational guidance value for Washington, as recommended by DOH, is 6 µg/L. This recommendation has been reviewed and accepted by DOH's Scientific Advisory Committee.

DOH's recommendation may change in the future if EPA's DRAFT risk reference dose of 0.006 µg/kg-day (derived for short-term and subchronic exposure durations) is adopted after review (USEPA 2006a). Because EPA's risk reference dose has not been adopted and because it is inconsistent with the WHO TDI, DOH's recommended recreational guidance value is provisional and may be updated as new information becomes available or if federal guidelines are developed.

Some counties in Washington use cell densities to monitor lakes. DOH considered using cell counts in a state management approach, particularly for anatoxin-a, but did not include cell densities to trigger an advisory because Ecology does not fund cell counts, only toxicity tests. At the county level, a local health jurisdiction (LHJ) may follow WHO guidelines and post a lake if the cell density of total toxigenic cyanobacteria equals or exceeds 100,000 cells/mL, unless the bloom has *Microcystis* or *Planktothrix* species, in which case the lake would be posted if there are 20,000 cells/mL. The guidance value associated with *Microcystis* or *Planktothrix* cell counts is estimated to correlate with the production of 4 µg/L microcystin.

Anatoxin-a Risk Levels and Standards

Anatoxin-a is a potent acute neurotoxin. Available data indicate that health concerns based on chronic toxicity (vs. acute toxicity) of anatoxin-a would not be significant (WHO 1999). In 1999, WHO concluded that the toxicity database is insufficient for derivation of a TDI for anatoxin-a. Metcalf and Codd (2004) report that further toxicity data via oral and lifetime exposure are needed to derive guidance values for remaining known cyanobacterial toxins, including anatoxin-a. Oral toxicity and lifetime-exposure studies are ideal to minimize uncertainties and have been determined only for microcystin-LR and recently for cylindrospermopsin. Because acute effects are the primary endpoint of concern for recreational exposure to anatoxin-a and data are not available for derivation of an acute RfD, DOH reviewed other options for use in recreational guidance and has recommended the most protective approach until an acute RfD is available to calculate an anatoxin-a guidance value.

A 2006 EPA DRAFT toxicological review of anatoxin-a concluded that available oral toxicity information is inadequate to support derivation of oral RfD values for acute and chronic exposure durations to anatoxin-a. The anatoxin-a database is limited in number and quality of studies on effects following oral exposure to sublethal levels. However, neurotoxicity and death were observed in acute, short-term, and subchronic oral animal studies (USEPA 2006b). The authors conclude in their draft report that available data are insufficient to derive acute and chronic oral RfDs.

Based on the above data, EPA (2006b) was able to derive a DRAFT short-term RfD of 3×10^{-3} mg/kg-day by dividing the no observed adverse effect level (NOAEL) by an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies). The NOAEL of 2.5 mg/kg-day was based on a systemic toxicity study in mice exposed to anatoxin-a for 28 days (Fawell and James 1994, Fawell et al. 1999). Using the equation:

$$\textbf{Guidance value (mg/L)} = \frac{\textbf{Short-term RfD} \times \textbf{BW}}{\textbf{IR}},$$ where

Short-term RfD = 0.003 mg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The resulting short-term recreational guidance value using the short-term RfD would be 0.45 mg/L (450 µg/L).

Additionally, EPA (2006b) derived a DRAFT subchronic RfD of 5 X 10-4 mg/kg-day by dividing the NOAEL by an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 for interindividual variability, and 10 for database deficiencies). The NOAEL of 0.5 mg/kg-day was based on systemic toxicity in rats exposed to anatoxin-a for seven weeks (Astrachan and Archer 1981 in EPA 2006, Astrachan et al. 1980). A short-term recreational guidance value was calculated as follows:

Guidance value (mg/L) = Subchronic RfD X BW, where
IR

Subchronic RfD = 0.0005 mg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The resulting subchronic recreational guidance value using the subchronic RfD would be 0.075 mg/L (75 µg/L).

As stated previously, neither guidance value calculated above is based on acute toxicity data. The endpoint of concern for anatoxin-a is the acute endpoint (frank effects or death). Acute toxicity data for anatoxin-a are limited to results of lethality assays in mice, with neurotoxicity identified as the cause of death and with a single dose LD_{50} value of 13.3 mg anatoxin-a/kg (Fitzgeorge et al, 1994, Stevens and Krieger 1991). Fawell et al. (1999) conducted studies to assess the risk of effects on man of ingestion of anatoxin-a at low levels over longer periods. Their study used *in vivo* and *in vitro* experiments to investigate subacute toxicity, teratogenicity, and pharmacology of anatoxin-a in the mouse. Repeated sub-lethal oral administration over 28 days did not produce any reliable evidence of treatment-related toxicity and the authors conclude that anatoxin-a does not appear to be a developmental toxicant in mice. In their conclusions, the authors indicate that a guideline value for anatoxin-a in drinking water of 1µg/L would provide an adequate margin of safety of about three orders of magnitude (Fawell et al. 1999).

In summary, DOH recommends the use of 1 ug/L to provide an adequate margin of safety of about three orders of magnitude, based on Fawell et al. (1999). This recommendation is a protective approach for use in the absence of an acute anatoxin-a RfD and was accepted by DOH's Scientific Advisory Committee. The recommendation of 1 ug/L will be updated when an acute RfD for anatoxin-a is available to calculate a guidance value.

Washington Lakes: Three-Tiered Approach to Managing Lakes with Cyanobacterial Blooms

The following framework for managing toxic or potentially toxic cyanobacterial blooms uses a three-tiered approach. The framework applies the recreational guidance values derived above (6 µg/L microcystins and 1 µg/L anatoxin-a) and is recommended for managing Washington lakes (Figure 1).

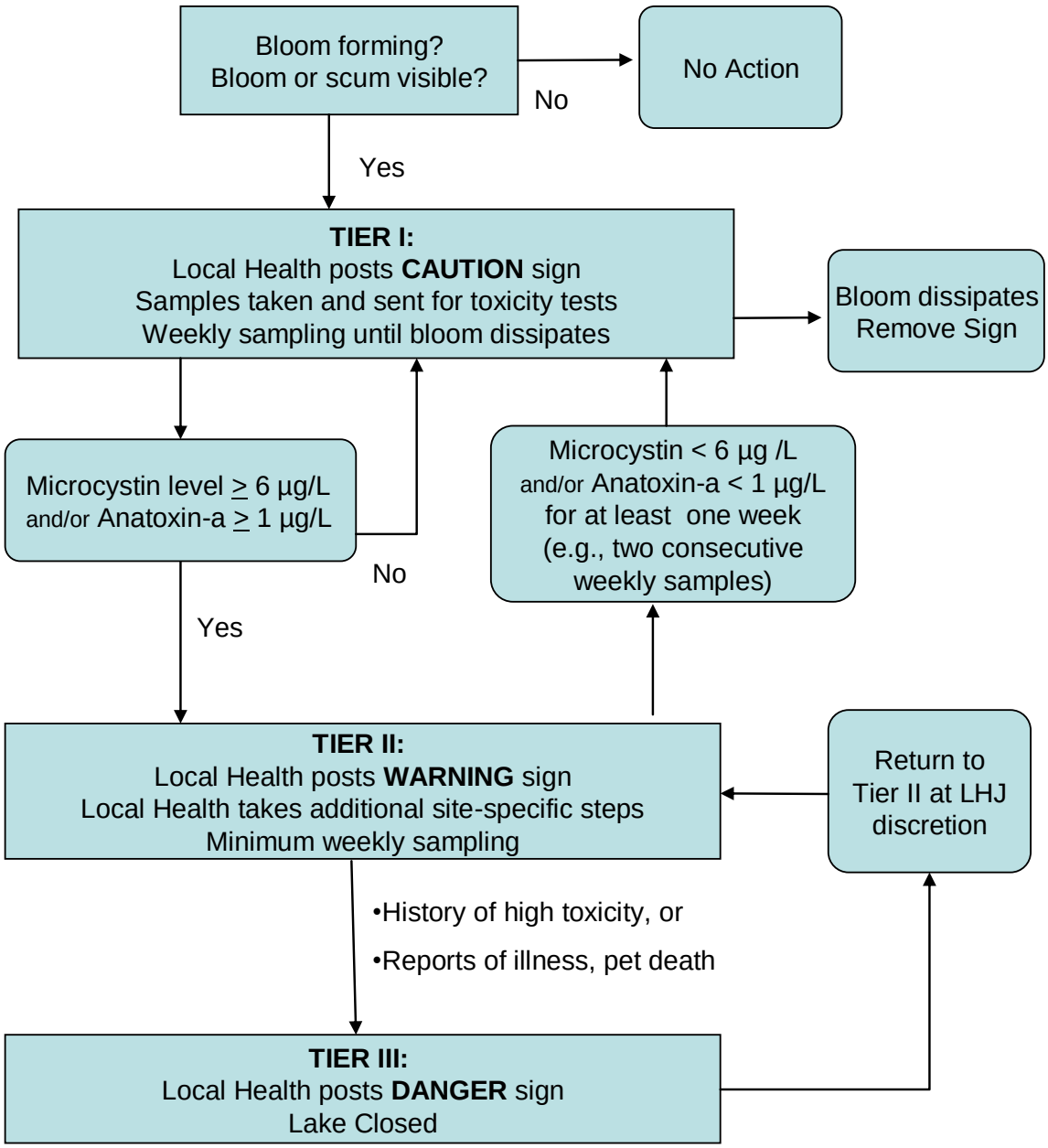
A unique feature of the Washington approach to cyanobacterial blooms in lakes is that bloom samples from all water bodies are eligible for toxicity testing. DOH and Ecology have incorporated outreach and educational efforts to encourage local health jurisdictions (LHJs), other agencies, lake residents, and the general public to notify Ecology or LHJ when a potential bloom is observed. The reported incidence of blooms may be associated more with the circumstance of observation than any other single factor.

Observers should look for developing blooms and surface accumulations which can occur in any nutrient-rich water such as lakes, ponds, or river embayments. Although conditions needed for a bloom to form or produce toxin are complex and uncertain, elevated nutrients such as nitrogen and phosphorous, pH 6-9, and favorable temperatures support cyanobacteria growth. Weather conditions can influence the area of biomass accumulation. For example, intense rain or heavy wind conditions mix water so that surface accumulations may not be evident. Also, a steady, light wind may blow surface waters toward a given shore and cause biomass accumulations. Because scum formations and blooms are extremely transitory in nature and highly influenced by wind patterns, it is challenging to quantify toxin levels from one or two samples unless a scum covers the entire lake.

Upon notification of a potential bloom, the LHJ or other agency staff (or lake resident) will obtain a sample number from Ecology, sample the water body experiencing the bloom, then send the sample to the laboratory for toxicity tests. Sampling and shipping directions are available from Ecology's Freshwater Algae Control Program: http://www.ecy.wa.gov/programs/wq/plants/algae/monitoring/index.html.

At present the King County Environmental Laboratory (KCEL) is under contract with Ecology to test for microcystins and anatoxin-a. Results of toxicity analyses will be incorporated into Ecology's database and accessible via Ecology's website.

Figure 1. Three-tiered approach to managing Washington water bodies with cyanobacterial blooms.



Tier I

A sample of a visible cyanobacteria bloom or scum is sent for phytoplankton examination and toxicity testing. Results are sent to the appropriate LHJ, to the agency that sent in the sample, and posted to Ecology's Freshwater Algae Program list serve. If the sample is dominated by potentially toxic cyanobacteria, the LHJ should post a CAUTION sign (Figures 1, A1). A

CAUTION advisory is intended to provide the public with information that a public health hazard might exist. In Washington, local jurisdictions have the authority to post advisories on water bodies within their districts (RCW 70.05.070).

If the bloom dissipates, the LHJ should remove the sign. If the bloom remains but microcystin levels are below 6 µg/L and/or anatoxin-a levels are below 1 µg/L, the CAUTION sign should remain posted. If the sample contains microcystin values equal to or greater than 6 µg/L and/or anatoxin-a values equal to or greater than 1 µg/L, move to Tier II.

Tier II

If microcystin levels are 6 µg/L or higher and/or anatoxin-a levels are 1 µg/L or higher, the LHJ should post a WARNING sign (Figures 1, A2). The lake should be sampled weekly, at a minimum, with the WARNING sign posted as long as microcystin concentrations are 6 µg/L or higher and/or anatoxin-a levels are 1 µg/L or higher. DOH recommends that LHJs wait one week once microcystin levels fall below 6 µg/L and/or anatoxin-a levels fall below 1 µg/L before retracting an advisory.

Toxin levels may be at their highest during bloom die-offs even though the water looks "normal." Another consideration is that toxin levels may be significantly lower due to temporary changes in weather conditions rather than changes in the cyanobacteria population. Heavy wind and/or intense rainfall can redistribute cyanobacteria throughout the lake and throughout the water column with little change in the total number of cyanobacteria cells. This makes it difficult to assess whether a bloom is declining or not. Therefore, DOH recommends that LHJs do not lift advisories unless they check the lake under weather conditions that are conducive with biomass accumulation (relatively calm or a light steady wind and little or no rainfall).

At this point, the LHJ might want to take additional steps in communicating risk, depending on severity of the bloom and historical use of the lake (i.e., a highly used access point such as a dog park might warrant greater outreach efforts as compared with a lake not known for any recreational activity). Time of year is another factor to consider since there is usually much less human recreational activity in Washington lakes in the winter due to lower temperatures. One possible risk communication tool is a press release by the LHJ. Another would be to notify local veterinarians and fish and wildlife officials so that they may issue information regarding the bloom. In certain situations, some LHJs have mailed notifications to local lakefront residents after confirmation of cyanobacterial toxicity. Other possible measures used to reach lakefront residents include radio messages or the internet via a list serve or "blast" email. DOH anticipates that successful LHJ outreach efforts may be duplicated in other counties as results of successful efforts become known.

If a lake has a history of high toxicity, if toxin concentrations are extremely high, or if pet illnesses/death or human symptoms are reported, move to Tier III. Implementation of Tier III is based on judgment of the LHJ and local knowledge of the water body.

Tier III

Under certain circumstances, a LHJ may wish to close a lake with unusually high microcystin or anatoxin-a concentrations. At the discretion of the LHJ, a water body can be posted as DANGER – Closed (Figures 1, A3). Examples include:

- very dense blooms covering an entire lake
- confirmed pet illnesses or death
- reported human illness.

The LHJ should post a press release to notify the general public of a lake closure. Also, LHJs should follow whatever additional methods of outreach, including those listed under Tier II, that will best inform public beach users and lake front residents of the risks from cyanotoxins and how to avoid these risks.

Retraction of a lake closure is also at the discretion of the LHJ. DOH recommends posting a WARNING sign and following Tier II recommendations after retracting a lake closure until microcystin levels are less than 6 µg/L and/or anatoxin-a levels are less than µg/L (Figure 1).

Ecology's Freshwater Algae Control Program will add microcystin and anatoxin-a results to their website database as results are received from the laboratory. For Tier II and Tier III, actions taken by the LHJ such as posting or closing a lake will be published on the website and posted on Ecology's list serve.

Risk Perspective

Recognizing that local health jurisdictions may not have sufficient funds to enable the level of effort described in these guidelines, DOH would like to provide some perspective on the relative public health importance of cyanobacteria monitoring activities. It is beyond the scope of this document to compare or rank a relative risk of environmental contaminant exposures such as cyanotoxins, PCBs, lead, or mercury. We do know that exposure to cyanobacterial toxins may cause symptoms ranging from skin irritation to gastrointestinal upset to neurological problems to death. In the recent past, toxic blooms in Washington lakes have led to the death of small and large animals. DOH is concerned about potential impacts on humans and pets after short-term exposure to the nerve toxins and on impacts to humans and pets from long-term exposure to the liver toxins.

While potential impacts to public health from cyanotoxins are high, associated costs for sampling and toxicity tests are low. Ecology has provided financial assistance to cover the expense of microcystin and anatoxin-a toxicity testing through the Freshwater Algae Control Program. A limited number of microcystin sample kits (including mailers) are available for free from DOH. At this time, a larger sample size (and kit) is needed for anatoxin-a tests than for microcystins; this kit is not available from Ecology or DOH. In summary, LHJ costs would include staff time to sample a potential toxic bloom, cost of sample jars for anatoxin-a samples, and cost of mailing sample kits to the laboratory.

Summary

This document offers a recreational guideline value for microcystins calculated as follows:

$$\textbf{Guidance value (µg/L)} = \frac{\textbf{TDI X BW}}{\textbf{IR}}\textbf{, where}$$

TDI = 0.04 µg /kg-day
BW = 15 kg child
IR = 0.05 L/h, assuming 2 h/d.

The resulting (provisional) recreational guidance value of 6 µg/L (microcystins) is used in a three-tiered framework for LHJs or the appropriate local agency to follow in the event of a toxic bloom.

DOH recommends an interim (provisional) recreational guidance value of 1 ug/L anatoxin-a. This recommendation provides an adequate margin of safety of about three orders of magnitude and is based on a systemic toxicity study in mice exposed to anatoxin-a for 28 days (Fawell et al. 1999). When an acute RfD becomes available, DOH will reassess this interim anatoxin-a guidance value. The interim (provisional) recreational guidance value of 1 ug/L anatoxin-a is also used in a three-tiered lake management framework.

When a cyanobacterial bloom is developing or a bloom or scum is observed, LHJs may post CAUTION signs while samples are being tested for toxicity (Tier I). The CAUTION signs remain posted until the bloom dissipates (if microcystin levels remain below 6 µg/L and/or anatoxin-a levels remain below 1 µg/L).

If microcystin levels are above 6 µg/L, the LHJ posts WARNING signs until levels fall below 6 µg/L for at least one week (Tier II). Similarly, if anatoxin-a levels are above 1 µg/L, the LHJ posts WARNING signs until levels fall below 1 µg/L for at least one week (Tier II). LHJs may conduct additional outreach efforts as needed. Under rare circumstances, a LHJ may need to post DANGER signs (Lake Closed), which revert to WARNING signs at the LHJ's discretion.

Ecology and DOH personnel are available to discuss results of lake testing and consult with LHJs during their decision-making process. As part of statewide outreach efforts, DOH will coordinate with Ecology and local agencies to post advisories under the BEACH program's website. Ecology will update and maintain lake toxicity data on its Freshwater Algae Control Program website and list serve. Finally, each agency has additional information on freshwater algae and toxicity issues available on its website as a public education and risk communication resource.

References

Astrachan, NB and B.G. Archer. 1981. Simplified monitoring of anatoxin-a by reverse-phase high performance liquid chromatography and the sub-acute effects of anatoxin-a in rats. In: The Water Environment: Algal Toxins and Health, W.W. Carmichael, Ed. Plenum Press, New York, NY. p. 437-446.

Astrachan, N.B., B.G. Archer, and D.R. Hilbelink. 1980. Evaluation of the subacute toxicity and teratogenicity of anatoxin-a. Toxicon. 18(5-6):684-688.

Botana, L. 2007. Phycotoxins. Chemistry and Biochemistry. Blackwell Publishing. ISBN-13: 978-0-8138-2700-1. 345 pp.

Carey, C, J. Haney, and K. Cottingham. 2007. First Report of microcystin-LR in the cyanobaterium *Gloeotrichia echinulata*. Environmental Toxicology. Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/tox.20245.

Falconer, I. 2005. Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. CRC Press. ISBN 0-415-31879-3. 279 pp.

Fawell, J.F and H.A. James. 1994. Toxins from blue-green algae: Toxicological assessment of anatoxin-a and a method for its determination in reservoir water. FWR Report No. FR0492/DoE372.

Fawell, J.K., R.E. Mitchell, R.E. Hill, and D.J. Everett. 1999. The toxicity of cyanobacterial toxins in the mouse: II Anatoxin-a. Hum. Exp. Toxicol. 18(3):168-173.

Fitzgeorge, R., S. Clark, C. Keevil. 1994. Routes of intoxication. In: Codd, G.A., Jefferies, T.M., Keevil, C.W., Potter P., eds. Detection methods for cyanobacterial toxins. Cambridge, Royal Society of Chemistry. Pp. 69-74.

Gorham, P.R. (1964). Toxic algae. In: D. F. Jackson (ed.) Algae and Man. Plenum Press: New York, pp. 307-336. 434 pp.

Huisman, J., H. Matthijs, and P. Visser. 2005. Harmful Cyanobacteria. AQEC 3. Springer. Netherlands. ISBN 1-4020-3009-6. 241 pp

MacKintosh, C., K.A. Beattie, S. Klumpp, P. Cohen, G.A. Codd (1990). Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2a from both mammals and higher plants. Federation of the European Biochemical Society Letters. 264:187-192.

Merck Veterinary Manual. 2008. Merck & Co., Inc. Whitehouse Station NJ, USA

Metcalf, J.S. and G.A. Codd. 2004. Cyanobacterial Toxins in the Water Environment. FR/R0009. Foundation for Water Research. 36 pp.

Stevens, D, and R. Krieger. 1991. Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-a. Toxicon. 29(1):134-138.

Stone, D. and W. Bress. 2007. Addressing Public Health Risks for Cyanobacteria in Recreational Freshwaters: The Oregon and Vermont Framework. SETAC. (3)1:137-143.

USEPA. 2006a. Toxicological Reviews of Cyanobacterial Toxins: Microcystins LR, RR, YR and LA (External Review Draft). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/139, 2006.

USEPA. 2006b. Toxicological Reviews of Cyanobacterial Toxins: Anatoxin-a (External Review Draft). U.S. Environmental Protection Agency, Washington, DC, NCEA-C-1743, 2006.

WHO. 1999. Toxic Cyanobacteria in Water. A guide to their public health consequences, monitoring and management. I. Chorus and J. Bartram, eds. Taylor and Francis. New York and London. 416 pp.

WHO. 2003. Guidelines for safe recreational water environments. Volume 1: Coastal and fresh waters. World Health Organization. Geneva. 219 pp.

Appendix A

Caution, Warning and Danger Signs:
A Three-Tiered Approach

Figure A1. Caution sign for use in Tier 1.

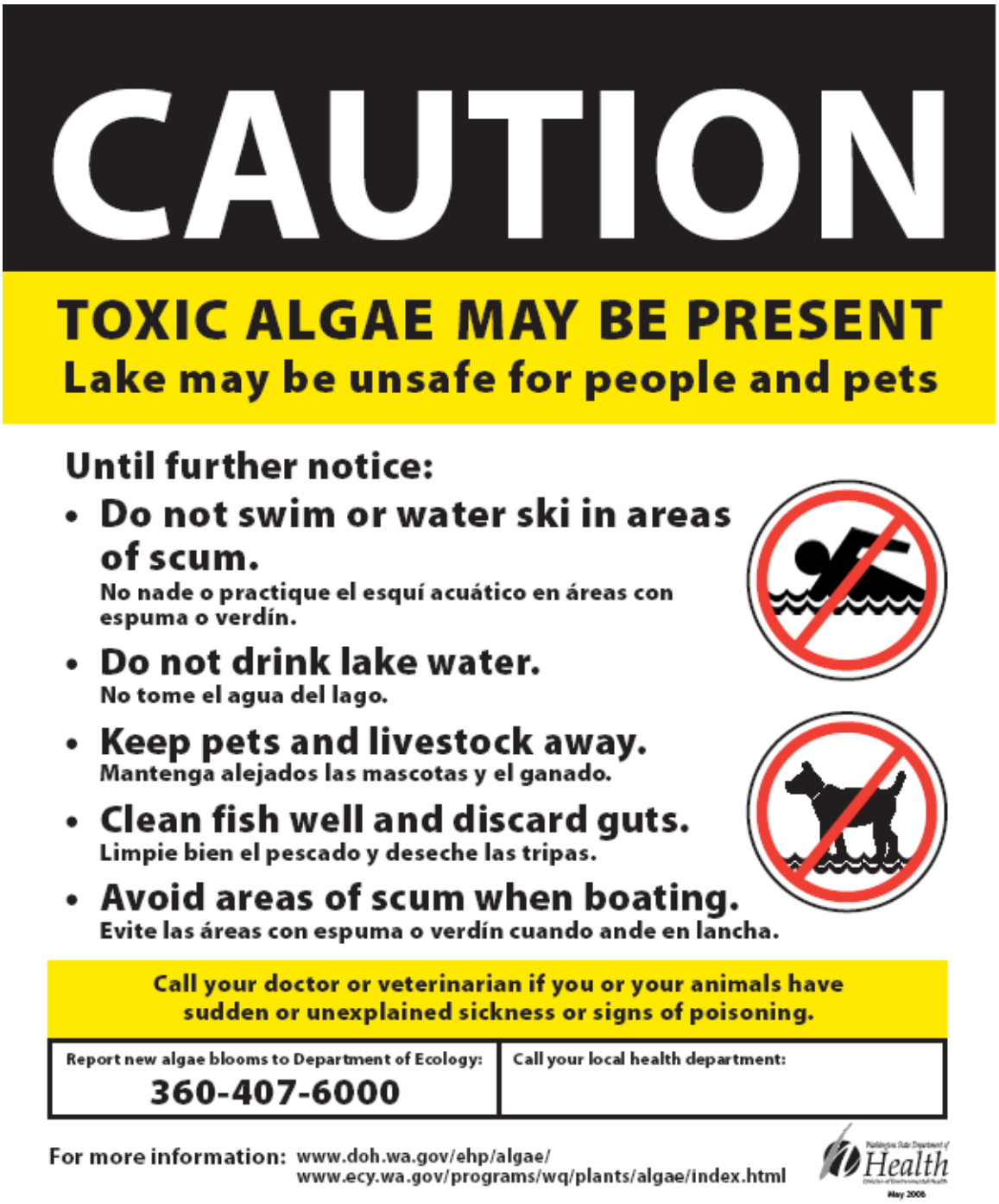


Figure A2. Warning sign for use in Tier II.

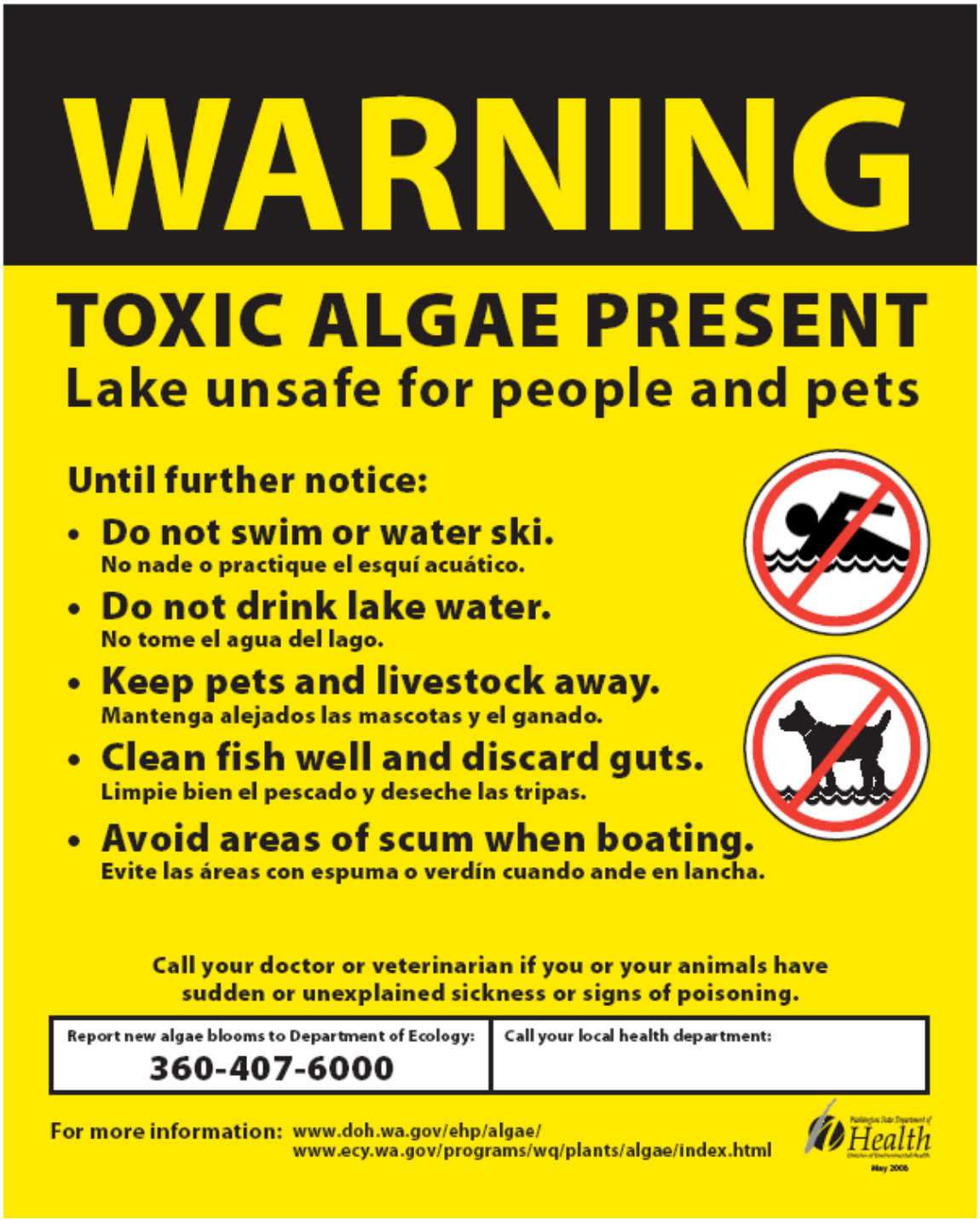


Figure A3. Danger sign for use in Tier III.